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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 19 OCTOBER 1961 No. 10

WEIGHT REDUCTION AND ITS SIGNIFICANCE

One of the series of diet conferences which was held in June, 1961 at the meetings of the American Medical Association in New York City was concerned with weight reduction and its significance. These were planned and sponsored by the Council on Foods and Nutrition of the A.M.A. and The Nutrition Foundation, Inc.

The first phases of the conference were directed at discussion of "Who is Obese?" Focus was given by a presentation of Dr. Richard Gubner of the Equitable Life Assurance Society of the United States. The claim was made that fatness is not a sign of health but rather contributes importantly to bodily ills and health risks as demonstrated by actuarial experience. Evidence was cited indicating that modern American women take their weight more seriously than do men. The average weight of women is about five pounds less than it was a generation ago. In contrast the average weight of men has increased about five pounds. There is little to indicate that obesity has an unfavorable influence on the mortality experience of younger women. However, in general women suffer the same hazards of obesity as men, although in lesser degree. Mortality rates for women are only about one-half those of men up to age 70, even among groups of obese men and women. The most marked influence of obesity in increasing mortality rates occurs in men in the earlier decades of adulthood and relates most importantly to deaths from coronary artery disease and diabetes mellitus. It was concluded, therefore, that the most serious hazards of obesity occur in younger men but that it is a problem which also confronts older women.

The problems of evaluating body weight as a measure or index of obesity were discussed. It was noted that tables of ideal weight which provide a range of weight for persons of given sex, height, and body frame are difficult to use because of the wide range in body weight specified. It was suggested that these tables might be adjusted for more effective use by indicating weight 10 per cent less than average for persons of the same age and height or by simply designating as ideal weight the average weight of persons when body growth is complete or at age 25.

Ideally, the determination of obesity should be in relationship to body fat content. There are crude but not precise relationships between body weight and body fat. Assessment of fat content of the body of American males indicates that the average content is about 10 per cent at age 20 and 23 per cent at age 55, while in the female values are about 20 per cent in groups 20 years of age and 30 per cent in those 55 years of age. Determination of body fat with precision requires use of laboratory methods to measure body density or distribution of certain gases. Less precise assessment can be made by determining the thickness of subcutaneous fat layers by x-ray or skin-fold thickness.

There is a great need for a simple procedure which might be applied by the person to himself. Dr. Gubner proposed the use of an index which he believes is easily derived and is applicable. He called this the "Perfect 36 Index." It is obtained by subtracting from the height as measured in inches the circumference of the waist in inches. In men height is recorded with shoes, in women without shoes. For both men and women a

value of 36 to 40 represents a desired range, while values above 40 indicate underweight, and appreciably below 36 represent obesity. In moderate obesity the index is usually in the range of 25 to 30, while values less than 25 correspond to marked obesity. It was not claimed that this index affords a direct or accurate measure of body fatness it is believed to be useful.

Dr. Vincent P. Dole of the Rockefeller Institute for Medical Research introduced a discussion of formula diets in weight reduction. He reviewed his eight years of experience with diet formulas in achieving weight reduction in a group of 48 patients, most of whom were markedly obese. Formulas of different composition were tested by isocaloric substitution, the protein content varying from 10 to 30 per cent of total calories. No differences were found in rate of weight loss, quantity of nitrogen lost, speed of regaining nitrogen equilibrium, liver function tests or depression in metabolic rate. The formula which was selected for usual use in weight reduction had a composition like that of human milk. It proved safe and acceptable as the exclusive food for patients observed on a metabolic ward for as long as 24 months.

Of the 48 obese persons who had lost from 20 to 170 pounds while under treatment, none had stayed at the reduced weight for more than a few months after discharge. Most of the patients had regained all of the lost weight during the five to eight year interval since discharge, and many had gone through several subsequent cycles of reduction and regain. No reduced person was able to eat normally without regain. This experience with markedly obese patients confirms the reports of other clinical investigators who have continued observations of obese patients over periods of years.

On the basis of this experience Dr. Dole takes issue with a number of widely held concepts concerning obesity. For instance, he does not believe that an obese patient can expect after weight reduction to stay thin on a diet equal in caloric value to the usual diet of a normally thin person. He has found that the total energy expenditure of markedly overweight subjects after weight reduction may be only two-thirds or half that of normally thin persons of comparable age, height, and sex. Such persons eating the same quantity of food as normal persons regain weight. He has not obtained evidence that metabolic rates increase to normal as the reduced fat person gradually adapts to a normal life.

Issue was taken also with the concept that all obese persons remain fat through persistent overeating (*i.e.*, eating more than normally thin persons). It was proposed that there may exist in the markedly obese a defect in fat metabolism at the cellular level that leads to the accumulation of fat in adipose tissue and accounts for the ready return to the prior state of obesity after weight reduction.

It was not agreed either that the main task of the physician is to reduce the weight of every obese individual. It was proposed that the first responsibility of the physician is to assist the patient to lead an active and productive life despite his handicap, relieving him so far as is possible of the shame that has been attached to this abnormality. The physician should encourage his overweight patients to increase their physical activity; this not only increases their expenditure of energy but may well have other favorable effects on fat metabolism. Indeed, most of the moderately obese middle-aged people of sedentary habits appear to suffer mainly from underactivity. For them the remedy is regular muscular exercise rather than starvation. Dietary management was accorded only a secondary place in treatment of the overweight. The ultimate objective in dieting should be to achieve a simple diet of plain foods suitable for long term use. The use of formula diets may serve in special cases as an adjunct either in initiating dietary restriction or checking periods of regaining weight. There would seem to be no

reason other than those of metabolic investigations in hospital settings to prescribe formula diets for prolonged periods of time for obese patients.

Dr. Herbert Pollack of New York City described the "module plan" for programs of weight reduction. In simplest terms this procedure attempts to utilize exercise and physical expenditure of energy as well as reductions in food consumption to effect calorie deficits. The energy value of each pound of body fat is 3,500 calories. Consumption of food in excess of need or reduction in energy expended to afford positive energy balance equaling 3,500 calories would account for a gain in body weight of one pound. Similarly, decreasing intake or increasing energy expenditure by a similar amount should lead to loss of one pound of body weight. If one plans in terms of modules of 50 or 100 calories per day represented either by calories expended in work or exercise or food removed from the daily diet, it is possible to adapt patterns of eating and living so that weight reduction may be achieved in amount and at a rate desired. The application of the module type of dietary approach does not require any detailed knowledge of food portions. It is based on the concept that one does not have to know exactly what the total caloric intake of an individual is. It is the creation of the deficit which brings about the desired reduction in body weight.

The conference considered the use of anorexogenic drugs for purposes of weight reduction. Dr. Martin G. Goldner of the Jewish Chronic Disease Hospital of Brooklyn emphasized that drug treatment can never replace dietary restrictions; it can only help the patient to adjust to the reducing program and to adhere to his diet. The available anti-obesity drugs can be classified into four groups, according to their main mode of action: those which increase expenditure of energy, those which induce negative water balance, those which cause incomplete digestion or absorption of food, and those which reduce the ingestion of food. Only those in the latter group deserve the name "anorexogenic drugs." Many of the anti-obesity drugs contain several agents with one or two of these modes of action.

Dr. Goldner reviewed the more than 80 drugs listed as anti-obesity agents in the current edition of the Physicians Desk Reference. Seven of them are metabolic stimulants; six diuretics and purgatives; eight intestinal bulk producers; 53 appetite depressants; and seven of miscellaneous types. All of the appetite depressant drugs are sympathomimetic amines (amphetamine or its cogeners). All are stated to be active through centers in the hypothalamus, either stimulating the ventromedial nuclei (satiety center) or suppressing the hypothalamic feeding center in the lateral nuclei. Thus, their action, in principle, is one of reducing caloric intake or enforcing dietary restriction. Serious side effects of these drugs are rare and few, but in all, the anorectic effect is coupled with the known stimulating effects of the amphetamines. Characteristically, with all of them, there is loss of activity or habituation with continued and prolonged use.

Nearly all of the clinical evaluations of effectiveness of these agents published in the medical literature indicate failure when the use of the drugs was not coupled with dietary restriction. Few of the clinical trials have tried to utilize a placebo to obtain clear-cut objective control of therapeutic benefit. Selection of a suitable placebo is difficult or impossible, since all of the anorexogenic drugs have a stimulant or benzedrine-like effect. There seemed to be agreement that although anorexogenic drugs are available in relatively great numbers and are undoubtedly prescribed frequently, their contributions to weight reduction are limited and not of great value. When prescribing these agents, the physician should understand that he is recommending treat-

ment which is not directed at the major problems in obesity. He must be simultaneously working to achieve understanding and application of principles of planned exercise and limitation of caloric intake in his patient which will bring about the energy deficits with which body fat is burned and body weight reduced. Anorexogenic drugs may at best take the hunger pangs out of dieting.

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EFFECTS OF CHOLESTYRAMINE, A BILE ACID SEQUESTERING EXCHANGE RESIN

Administration of cholestyramine relieves the itching of incomplete biliary obstruction, leads to reduction in serum cholesterol concentration and, in larger doses, interferes with fat digestion, causing steatorrhea.

Several recent articles in this journal have reviewed research on the reduction of serum cholesterol by nondietary means: administration of the thyroxine analogue tetraiodothyroformic acid (*Nutrition Reviews* **19,** *209* (*1961*)), "SKF-525A" (*Ibid.* **19,** *251* (*1961*)), and triparanol (*Ibid.* **19,** *262* (*1961*)). The effectiveness of the first is doubtful. The last two are inhibitors of cholesterol synthesis. Undesirable and metabolically interesting side effects have been reported from drugs of this class, including fatty liver, skin and hair damage, and inhibition of adrenal steroid secretion (*loc. cit.*).

A different approach was used by D. M. Tennent *et al.* (*J. Lipid Research* **1,** *469* (*1960*); abstract in *Circulation* **20,** *969* (*1959*)). They reasoned that sequestration of bile acids in the intestinal tract by a suitable exchange resin, by preventing their reabsorption, would stimulate the oxidative destruction of cholesterol, which appears to be regulated by the need for bile acids in the enterohepatic cycle. They fed such resins to normal and to cholesterol-fed cockerels and to normocholesterolemic dogs and achieved substantial reduction in plasma cholesterol levels in all groups.

S. S. Bergen, Jr., T. B. Van Itallie, Tennent, and W. H. Sebrell extended the work to 26 human subjects (*Proc. Soc. Exp. Biol. Med.* **102,** *676* (*1959*)) with various diagnoses and serum total cholesterol levels of 133 to 400 mg. per 100 ml. They administered 13 to 15 g. of the anion exchange resin cholestyramine ("MK-135") per day, in an oral suspension, for ten to 236 days. The average of serum total cholesterol values during treatment, for each patient, fell from 6 to 38 per cent. Minimum values were appreciably lower. Levels usually began to fall within four days of the start of treatment and rose promptly after the resin was stopped. The principal side effect was constipation.

S. A. Hashim and Van Itallie later reported "dramatic" relief of long-standing intractable itching in three jaundiced patients with primary biliary cirrhosis and generalized xanthomatosis treated with cholestyramine (*J. Invest. Dermat.* **35,** *253* (*1960*)). The itching of obstructive jaundice has long been attributed to the accumulation of bile acids in the skin.

In an overlapping series of four similar patients (*Fed. Proc.* **20,** *248* (*1961*)) Hashim and Van Itallie observed serum total cholesterol concentrations, before treatment, of 712 to 1,478 mg. per 100 ml. and serum total bile acids of 10.4 to 14.2 mg. per 100 ml. Cholestyramine was administered for one to 14 months, with marked relief of pruritus. Total serum cholesterol levels decreased in three cases by 43 to 66 per cent and serum

bile acid concentrations decreased in all cases, to 1.9 to 6.2 mg. per 100 ml.

J. B. Carey and G. Williams (*J. Am. Med. Assn.* **176,** *432 (1961)*) likewise found that cholestyramine relieved itching in one jaundiced patient with biliary cirrhosis and in three with metastatic carcinoma of the liver, with concomitant falls in serum bile acid concentrations. By reducing the dose of resin the authors were able to demonstrate relief from itching in the first patient with as little as 1 g. of resin per day. A fifth patient, with carcinoma of the pancreas, was considered to have virtually complete obstruction of the common bile duct. Seven days of resin treatment produced, as expected, no change in serum bile acid concentration or in pruritus in this patient. Stool bile acid was increased about eightfold (from 31.2 to 245 mg. per day) in one healthy volunteer subject taking 10 g. of cholestyramine per day. The predominant bile acid was identified as deoxycholic acid.

In another paper Hashim, Bergen, and Van Itallie showed that cholestyramine, in larger doses, can sequester bile acids sufficiently to cause steatorrhea (*Proc. Soc. Exp. Biol. Med.* **106,** *173 (1961)*). They studied, in all, 16 healthy adults from 24 to 32 years old. Fat balance studies were performed on four subjects maintained on formula diets. Fat absorption, with I^{131}-labeled triolein technique, was measured on five subjects and fatty acid absorption on seven.

Two subjects showed no significant increase in fat excretion when given 15 g. of resin per day. Two others, given 30 g. per day, showed gross steatorrhea, with stool fat contents of four to five times the amounts excreted during the control periods.

In five subjects given a regular diet plus 30 g. of resin daily, absorption of labeled triolein was impaired. Seven subjects given the regular diet plus the same dose of resin absorbed labeled oleic acid normally.

The authors conclude that cholestyramine, by sequestering the bile acids, interferes with the digestion of fats, but that fatty acid absorption is unimpaired. They believe the "malabsorption" to be innocuous.

Thus it appears, from the evidence so far available, that sequestration of intestinal bile acids by the exchange resin cholestyramine may be a much safer means of reducing the serum cholesterol concentration than is the use of inhibitors of cholesterol synthesis. In addition, the resin is of real value in relieving pruritus associated with jaundice, if biliary obstruction is not complete.

Apparently, since fat digestion is unimpaired on doses of 15 g. of the resin per day, one need not anticipate interference with the absorption of fat-soluble vitamins; however, this remains to be proved. With increased oxidative destruction of cholesterol one would expect increased formation; the possible metabolic effects of this are unexplored. Since cholestyramine is neither absorbable nor digestible, systemic toxic effects are scarcely possible and one would not expect side effects comparable to those which make the inhibitors of cholesterol synthesis undesirable for clinical use.

DIETARY CHOLESTEROL AND SERUM LIPIDS IN MAN

Ingestion of cholesterol in the form of egg yolk elevated the serum level of cholesterol, phospholipid, and triglycerides. Removal of cholesterol from the diet depressed the level of these lipids.

The relationship between dietary cholesterol and the genesis of atherosclerosis in man has been investigated by numerous groups (*Nutrition Reviews* **18,** *9 (1960)*). It is well known that practically all experimental diets designed to produce athero-

sclerosis and hypercholesteremia in animals include added cholesterol. However, in the case of man, there is considerable doubt whether dietary cholesterol contributes to the level of serum cholesterol (A. Keys *et al.*, *J. Nutrition* **59,** *39 (1956)*).

Many previous investigators in this field administered cholesterol to subjects in one of two forms, either as crystalline cholesterol or as specific natural foods high in cholesterol, such as egg yolk. It has been noted that large amounts of crystalline cholesterol fail to increase the blood cholesterol levels but that cholesterol in the form of egg yolk may do so. In many of the studies, in addition to including cholesterol, the diets were so adjusted that the amount and type of fat the patient received was considerably different from that given the control. Also, in strict vegetarian diets the serum cholesterol is usually low; however, such a diet differs from that normally consumed in this country.

Recently, W. E. Connor, R. E. Hodges, and R. E. Bleiler (*J. Lab. Clin. Med.* **57,** *331 (1961)*) have reinvestigated the effects of feeding and not feeding cholesterol to normal volunteers. The cholesterol given was present in commonly eaten foods. For their study they used six healthy men between the ages of 40 and 45. Four subjects were well nourished; two were 20 to 35 pounds overweight. Except for xanthelasma on the lower lids of one individual, physical and laboratory examination revealed them to be normal. The subjects remained in good health during the entire study.

The subjects were divided into two groups, A and B, each consisting of three men. Each group was studied during four different dietary periods. During the first period (35 days) all the men received a regular hospital diet. This interval allowed them to become familiar with the metabolic division procedures and to establish baseline levels. During the second period (75 days) Group A was given a high cholesterol diet and Group B was fed a cholesterol-free diet. The cholesterol intake of groups A and B was reversed during period three (23 days). During the fourth period (17 days) all subjects were allowed to choose their diet.

During the second period the men were maintained on a formula diet drunk from a cup. It was complete in all nutrients. As a source of fat the subjects received either egg yolk or peanut oil, those on the high cholesterol diet receiving egg yolk while those on the cholesterol-free diet received only peanut oil. The amount of protein in the diets varied: one pair of subjects received 20 g. per day; another pair received 1 g. of protein per kg. of body weight per day; the third pair received 2 g. of protein per kg. of body weight.

The three subjects who received the high cholesterol diet received 1,650, 1,900, and 4,800 mg. of cholesterol per day. The caloric intake and the relationship between saturated and unsaturated fatty acids were similar in both groups.

During the third period, when Group A was shifted from a high cholesterol to a cholesterol-free diet and Group B changed from a cholesterol-free to a high cholesterol diet, the subjects received diets consisting of natural solid foods; the cholesterol was supplied by egg yolk. During this time all subjects were given a diet high in protein, 114 to 139 g. per day. Again the fatty acid composition was similar in both diets. This similarity was accomplished by adjusting the dietary fat content, using peanut oil or egg yolk depending on the group. During the control period, the serum cholesterol of five of the subjects ranged from 200 to 248 mg. per 100 ml. while one subject had a mean value of 280 mg. per 100 ml. On the high cholesterol diet three subjects from Group A had mean increases of serum cholesterol varying from 25 to 36 mg. per 100 ml., while the three men on the cholesterol-free diet all had decreases in their serum cholesterol ranging from 44 to 84 mg. per 100 ml.

In addition to changes in the cholesterol, the serum levels of phospholipids rose in the group fed the high cholesterol diet and fell in that receiving the cholesterol-free diet. Serum triglyceride levels increased in two of the three subjects on the high cholesterol diet and decreased in the subjects on the cholesterol-free diet.

When the diets were reversed, the subjects of Group B who were previously fed the cholesterol-free diet and were now given the high cholesterol diet increased their serum cholesterol levels by 15 to 80 mg. per 100 ml. A much greater fall in the serum cholesterol levels was noted in all the subjects in Group A who changed from the high cholesterol to the cholesterol-free diet. The average decrease in the serum cholesterol levels ranged from 94 to 115 mg. per 100 ml. In addition, the phospholipids fell 34 to 65 mg. per 100 ml. and the triglycerides decreased from 17 to 131 mg. per 100 ml.

In the fourth period, when the general diet was fed, an increase in serum cholesterol of 55 to 77 mg. per 100 ml. occurred in the subjects of Group A (previously on a cholesterol-free diet). Two of the three subjects who shifted from the high cholesterol diet to the general free choice diet had decreases in serum cholesterol.

Subjects of both groups lost weight during the study; the investigators believe that weight reduction during the period of study was not a major factor in producing lowering of the serum cholesterol levels.

In this study, paired groups of individuals received different levels of dietary protein, which ranged from 20 to 143 g. per day. There was no relationship between the amount of dietary protein consumed and the serum cholesterol response. The nitrogen balances did not appear to have a direct effect on the level of cholesterol in the serum.

The range of cholesterol intake tested in the previous study (1,700 to 4,800 mg. per day) exceeded the daily American consumption of 500 to 1,000 mg. Accordingly, Connor, Hodges, and Bleiler (*J. Clin. Invest.* **40,** *894 (1961)*) designed further experiments to determine whether lower levels of egg yolk or crystalline cholesterol would also influence the serum cholesterol and phospholipid levels.

Six normal men were fed the test diets by stomach tube during a 12 week experiment. The fatty acid pattern of the egg yolk fat was simulated using a mixture containing 60 per cent peanut oil, 30 per cent cocoa butter, and 10 per cent safflower oil. Only the level of cholesterol was varied. Diets were changed every three weeks so that comparison could be made of the effects of feeding cholesterol-free and cholesterol-containing diets. Period 1 and 3 diets were cholesterol-free. Period 2 diets contained either 475, 950 or 1,425 mg. of egg yolk cholesterol daily. Period 4 diets contained either 1,200, 2,400 or 3,600 mg. of crystalline cholesterol daily. Serum total cholesterol, phospholipid, and triglycerides were determined twice weekly in each period.

The serum cholesterol of the subjects varied from 200 to 300 mg. per 100 ml. during ingestion of the general diet but diminished 58 mg. per 100 ml. after three weeks on the cholesterol-free regimen. Addition of the various quantities of egg yolk cholesterol resulted in essentially similar increases in serum cholesterol in all cases (66 to 77 mg. per 100 ml.). Removal of cholesterol (period 3) resulted in a drop of 55 mg. per 100 ml. in serum cholesterol. In period 4 the addition of crystalline cholesterol to the cholesterol-free ration resulted in an increase in the average serum cholesterol of 19 mg. per 100 ml. The average serum phospholipid diminished by 44 mg. per 100 ml. as a result of shifting from the general diet to the cholesterol-free diet of period 1. The average serum phospholipid increased by 42 mg. per 100 ml. as a result of feeding the egg yolk diet and diminished by 44 mg. per 100 ml. when the egg yolk diet was discontinued. Feeding crystalline cholesterol

resulted in an average increase of 22 mg. phospholipid per 100 ml. of serum.

Average serum triglycerides decreased (47 mg. per 100 ml.) when the cholesterol-free ration replaced the general diet, increased when egg yolk was given but remained unchanged during the cholesterol-free period 3 and the crystalline cholesterol period 4.

The concentration of lipids in the stool rose above normal limits only when crystalline cholesterol was included in the diet. Subject 5, for example, excreted 2.0 g. of fat on the cholesterol-free diet but during the feeding of 3.6 g. of cholesterol daily the stool fat increased to 5.2 g. daily.

The response of the serum cholesterol to the ingestion of crystalline cholesterol was less than the response to egg yolk cholesterol. This difference may have been due to the fact that egg yolk cholesterol is absorbed better (67 per cent compared to 11 per cent for crystalline cholesterol), according to the report of R. P. Cook, D. C. Edwards, and C. Riddell (*Biochem. J.* **62,** *225 (1956)*). In addition, the capacity of the organism to absorb cholesterol appears to be limited. The results indicate that the serum cholesterol increase was not significantly different whether the diet contained the equivalent of two egg yolks or six.

The investigators equalized the patterns of fatty acids of the two diet fats in an effort to make the diets more comparable. In spite of this attempt there was a difference in the serum cholesterol elevation which must have been unrelated to the fatty acid pattern per se. Other more subtle differences may have been influential in producing this difference in elevation of serum cholesterol.

The results of the two studies tend to complement each other. The latter study indicates that even with dietary cholesterol intakes similar to those frequently consumed by man, the influence on serum cholesterol is prompt and definite. From these studies one must conclude that ingestion of dietary cholesterol does elevate the serum cholesterol in man and probably should be considered as a potential factor influencing the pathogenesis of atherosclerosis.

PARENTERAL FLUID THERAPY IN INFANTILE DIARRHEA

Three methods of parenteral fluid therapy for the initial rehabilitation of infants with diarrhea were compared.

The art and practice of parenteral fluid therapy have been brought to a very high state indeed. The efficiency of this type of treatment is evident in the falling death rate from infantile diarrhea. Each of several methods for parenteral fluid therapy is highly praised by its proponent, and a rather spirited controversy has been in progress among them and their supporters for some two decades.

A. F. Hartmann and co-workers advocated the use of substantial amounts of sodium lactate, in the conviction that the acidosis of infantile diarrhea should be relieved promptly and completely (Hartmann and M. J. E. Senn, *J. Clin. Invest.* **11,** *327 (1932)*; Hartmann *et al.*, *J. Pediat.* **13,** *692 (1938)*; Hartmann, *Pediatrics* **2,** *584 (1948)*).

D. C. Darrow and co-workers found that large losses of potassium occurred during the course of diarrhea, and emphasized the liberal use of this ion while giving somewhat smaller amounts of sodium (Darrow, *J. Pediat.* **28,** *541 (1946)*; Darrow, J. Flett, Jr., and E. L. Pratt, *Pediatrics* **4,** *604 (1949)*; Darrow, *Ibid.* **9,** *519 (1952)*).

N. B. Talbot and A. M. Butler and co-

workers undertook to define the limits of tolerance which the sick infant's or child's homeostatic mechanisms would permit for the administration of water and electrolytes (Butler and Talbot, *New Engl. J. Med.* **231,** *621 (1944)*; Talbot *et al., Ibid.* **252,** *856 (1955)*). Their scheme called for dilute sodium solutions, very liberal amounts of potassium, and the addition of phosphate to the intravenous infusion.

At first glance these regimens would appear to be vastly different, yet clinical experience has shown that satisfactory results have been achieved in the treatment of infantile diarrhea through the use of each of them. When used injudiciously each has its hazards—that of alkalosis and potassium deficiency with the Hartmann method, potassium excess with the Darrow method, and water intoxication with the Butler-Talbot regimen.

H. L. Smith and J. N. Etteldorf (*J. Pediat.* **58,** *1 (1961)*) have undertaken a comparative study of these three regimens in a series of infants suffering from severe diarrhea. These infants were dehydrated on admission to the hospital. All were acidotic (average blood pH 7.15; average plasma bicarbonate content 10 mEq per liter). Plasma sodium concentration varied widely (122–194 mEq per liter) as did the concentration of potassium in the plasma (2.0–7.3 mEq per liter). In the latter two respects the series was far from homogeneous. No estimates of the degree of dehydration manifested by these infants (other than clinical appearances) were attempted by the authors. However, precise means for making such estimates are not readily available in most clinical situations.

The infants were cared for in an air-conditioned room. Parenteral fluids alone were given for the first 18 hours. Then small amounts of 5 per cent dextrose solution were given orally. After 48 hours the parenteral infusions were stopped and further rehabilitation achieved with dilute milk formulas. Samples of blood for determination of pH and plasma electrolytes were obtained initially and at about the fourteenth, thirty-eighth, and sixty-second hour of therapy. No attempt was made to study urinary or fecal losses of electrolyte or to calculate retentions. Assessment of the infants' progress was made by clinical observations and the response of blood pH and plasma electrolytes.

A total of 19 infants was studied on the Butler-Talbot regimen, 14 on the Darrow regimen, and 20 on a modification of the Hartmann regimen which included somewhat more potassium. No other parenteral fluids, such as whole blood or plasma transfusions, were given to these patients.

The plan for parenteral fluid therapy consisted of two phases, an initial phase of rapid fluid administration, lasting 90 minutes or less and designed to effect an immediate expansion of body fluid volume, and a subsequent reparative phase during which the infusion ran much more slowly.

For the initial phase the infants on the modified Hartmann regimen were given 30 ml. per kg. body weight of one-sixth molar sodium lactate, on the Butler-Talbot regimen 25 ml. per kg. of 0.33 per cent sodium chloride in 5 per cent dextrose, and on the Darrow regimen 30 ml. per kg. of 0.45 per cent sodium chloride in 5 per cent dextrose solution. Thus, the first group received two to three times as much sodium as the latter two, and of course, no chloride.

The best way to illustrate the differences in the three regimens is to sum up the total intake of water, carbohydrate, and various electrolytes given during the first 24 hours of therapy. Expressed on a per kilogram basis, these are as follows: for the Hartmann, Butler-Talbot, and Darrow methods respectively: water 200, 200, and 210 ml.; carbohydrate 5,10, and 6.5 g.; sodium 14, 8.4, and 12 mEq; potassium 1.4, 6.0, and 2.8 mEq; chloride 5.8, 8.4, and 10.7 mEq; lactate 10, 3.4, and 4.2 mEq; phosphate 0, 2.6, and 0 mEq (as $H PO_4$); and calcium 0.2, 0, and 0 mEq. Total osmolarity (ex-

cluding carbohydrate) was 158, 138, and 140 milliosmols per liter for the three methods, respectively. The differences are readily apparent.

What is sometimes overlooked by the champions of the respective methods, however, is the fact that there are many similarities. Each provides for an initial phase of relatively rapid hydration of the patient, each provides the same water load and a modicum of carbohydrate, and each results in the administration of an electrolyte solution of approximately one-half the osmolarity of the extracellular fluid. The major differences are in the ratio of individual electrolytes. Hartmann's method (as modified) provides an Na/Cl/K ratio of 1/0.4/0.1, the Butler-Talbot method 1/1/0.7, and the Darrow method 1/0.9/0.2. The small amounts of calcium provided in the Hartmann regimen and the phosphate given by Butler and Talbot probably are of little significance.

The results obtained by the three methods are about what one would predict. Under the modified Hartmann regimen the hyperpnea due to acidosis disappeared within a few hours, and the plasma bicarbonate content and blood pH rose promptly to normal. Correction of acidosis occurred much more slowly under the Butler-Talbot regimen. However, mild alkalosis was a frequent occurrence with the Hartmann method and levels of plasma potassium often fell to below normal, with plasma sodium values higher than normal; three patients developed edema.

The Butler-Talbot method quickly corrected hypokalemia, but a few patients had slight hyperkalemia (without symptoms) and many showed a tendency to hyponatremia. Results of the Darrow regimen generally were intermediate in character: acidosis was corrected less rapidly than by the Hartmann method, but there was no tendency towards alkalosis. Normal levels of plasma potassium were promptly attained without a tendency toward hyperkalemia.

It is of interest that the response of infants who were hypernatremic on admission to the hospital was somewhat slower than that of those who were hyponatremic, although eventually the response was satisfactory. The problems which such infants present in therapy are well known.

Smith's and Etteldorf's study, though conducted without certain refinements such as balance measurements, does serve to emphasize several important features in parenteral fluid therapy as applied to infantile diarrhea. First is the need of such patients of liberal amounts of water and electrolyte, made up in such a way that the resulting solution is hypo-osmolar. Second is the need for including potassium as well as sodium in the repair solution. Thirdly there must be a provision for giving sodium in excess of chloride. (Other important considerations, such as including carbohydrate, were not studied in detail by these authors.)

Lastly, this study emphasizes that a number of different regimens are rather satisfactory providing certain basic needs of the patient are met. Each regimen has its particular strong points and its disadvantages, yet the over-all picture is satisfactory. This fact is a testimonial to the Butler-Talbot school, which has emphasized so strongly the fact that the infant's own homeostatic mechanisms will make the necessary adjustments in body fluid volume and composition provided sufficient water and electrolyte are administered, and provided the amounts given fall within the limits of these homeostatic mechanisms.

Considering all factors, the Darrow regimen is perhaps superior to the other two. It is of interest that Smith and Etteldorf are now at work on a modification of their own which they predict will be even better.

Each of the pioneers in parenteral fluid therapy has left his mark in the form of

greater understanding of the underlying principles and objectives involved. Each has made a unique and important contribution to our knowledge. The present study has sought to discover the best qualities of each method.

DIVERTICULOSIS OF THE JEJUNUM WITH MACROCYTIC ANEMIA AND STEATORRHEA

Three cases of this rare syndrome are reported. Patients were improved following surgery or treatment with vitamin B_{12} and antibiotics.

One of the rare forms of steatorrhea is that associated with diverticulosis of the jejunum and macrocytic anemia. Since patients with this disorder can be treated, it seems desirable to call attention to the syndrome in the hope that more cases will be recognized.

A. A. Polachek, W. J. Pijanowski, and J. M. Miller have reported three new cases (*Ann. Int. Med.* **54,** *636 (1961)*). In reviewing the literature on jejunal diverticulosis the authors could find only 15 cases with all three components, eight with macrocytic anemia, and three with steatorrhea. One of these three cases has been discussed in this journal (*Nutrition Reviews* **19,** *138 (1961)*).

Polachek's group measured the absorption of radioactive vitamin B_{12} and that of I^{131}-labeled triolein in their patients.

Case I: A 64 year old Negro man had had fullness after meals from age 35 and intermittent vomiting at 54. Diverticulosis of the jejunum was diagnosed at 55 years; the hemoglobin at this time was 10.3 g. per 100 ml. The next year the patient complained of numbness. Macrocytic anemia was diagnosed, and signs of subacute combined degeneration of the spinal cord were found. The hemoglobin was 8.4 g. per 100 ml. There was free hydrochloric acid in the stomach and many megaloblasts in the bone marrow. The patient was successfully treated with vitamin B_{12}. At age 63 he returned with flatulence, belching, anorexia, and a 15 pound weight loss. The serum albumin was 2.4 and the globulin 4.8 g. per 100 ml. Radioactive fat absorption was "borderline low." Many jejunal diverticula were shown radiographically and subsequently 160 cm. of jejunum was excised, containing 65 diverticula from 1.5 to 6.5 cm. in diameter. The mucosa was "normal," the submucosa "infiltrated with many round cells."

Three weeks after operation, vitamin B_{12} absorption was much improved by the Schilling labeled vitamin B_{12} technique. The patient regained 15 pounds and for six months did not need vitamin B_{12} treatment. He died 19 months after operation, from hypertensive cardiovascular disease with uremia.

Postmortem examination showed multiple diverticula, 1 to 2 cm. in diameter, of the large and small intestine.

Case II: A 75 year old white woman was seen six years before death because of weakness, pallor, diarrhea and a 70 pound weight loss. Macrocytic anemia (hemoglobin 4.7 g. per 100 ml.) and low serum albumin (2.0 g. per 100 ml.) were found; there was free hydrochloric acid in the stomach. Numerous large diverticula of the small intestine were shown radiographically. There was hypoplasia of the red cell series in the bone marrow, but liver extract produced a good reticulocyte response of 15 per cent in five days. The patient stopped treatment and returned some months before death with a recurrence of her complaints and thrombophlebitis in one leg. Vitamin B_{12} absorption was very low; fat absorption was moderately impaired. After six days treatment with chloramphenicol and sulfathalidine, vitamin B_{12} absorption was much

improved (9.7 vs. 1.9 per cent in 24 hours; normal over 8 per cent). Treatment was unavailing and the patient died.

Numerous jejunal diverticula 4 to 5 cm. in diameter were found on postmortem examination as well as some large diverticula of the colon and bilateral pulmonary emboli, the cause of death.

Case III: A 76 year old white man had a history of weakness, fainting, and a 15 pound weight loss, six years before death. He was found to have a macrocytic anemia (hemoglobin 9.7 g. per 100 ml.) and low serum albumin (2.2 g. per 100 ml.). There was no gastric free acid after histamine. The bone marrow was megaloblastic. Multiple diverticula of the jejunum were found radiographically. Vitamin B_{12} produced a reticulocytosis of only 3 per cent and hematologic remission was slow.

Two years later the patient entered the hospital with cardiac failure, diarrhea, and a 12 pound weight loss. The red blood cell indices were then normal. Four years later the patient was readmitted because of diarrhea, emaciation, and confusion. The serum albumin was down to 1.6 g. per 100 ml., the globulin 3.6. Vitamin B_{12} excretion after the test dose was 0.4 per cent in 24 hours; after five days of treatment with tetracycline the excretion was 5.8 per cent, indicating much improved intestinal absorption. The absorption of I^{131}-labeled fat was impaired and not improved by pancreatin. Diarrhea stopped temporarily with tetracycline and cortisone treatment and the patient left the hospital. He died three months after his last hospital admission; an autopsy was apparently not done.

These three patients were 64 to 76 years old at death; all three had had symptoms for years, with extensive weight loss and severe hypoalbuminemia. Two or all three components of the syndrome were actually recognized six to nine years before death. In view of the results of medical or surgical treatment, one must conclude that these patients could have been greatly improved over a period of years had their disease been better understood.

The authors point out that all three patients had low absorption of labeled vitamin B_{12} and of labeled fat, hypoalbuminemia and "evidence of stasis" of gastrointestinal contents. Absorption of vitamin B_{12} improved in all three, in one after surgical removal of the diverticula and in two after antibiotic therapy.

Polachek's group believes that the defects in absorption in this group of patients are attributable to "stagnation in the diverticula, with bacterial overgrowth and inflammatory changes." In support of this theory they cite animal experiments in which blind loops or antiperistaltic loops of intestine led to macrocytic anemia.

The authors make a plea for better recognition of the syndrome. They conclude that the macrocytic anemia usually responds to treatment with vitamin B_{12} and broad spectrum antibiotics, and the steatorrhea and diarrhea to the antibiotics. They suggest that intestinal resection be considered only if medical treatment is unavailing.

ABSORPTION AND UTILIZATION OF NUTRIENTS IN MAN AFTER TOTAL GASTRECTOMY

Absorption and utilization of protein, fat, and carbohydrate after total gastrectomy is good in patients who have a jejunal pouch prosthesis to replace the stomach.

One condition which limits the use of total gastrectomy in the treatment of malignant neoplasm of the stomach is the poor nutritional status which often follows. Whether the impaired nutrition is the result of loss of the absorptive surface of the

stomach or is a secondary effect of voluntary decrease in food intake by the patient because of discomfort is not clear. Two common side effects of total gastrectomy are reflux esophagitis and dumping, both of which cause the patient a great deal of distress and are often severe enough to discourage the intake of appreciable amounts of food. It is not known whether nutrition would be adequate if the patient were able to accomodate physically the normal amount of food.

R. P. Hays and D. A. Clark (*Ann. Surg.* **152,** *864 (1960)*) have developed an operative procedure for making an ingenious food pouch in such patients, formed of three folds of jejunum, opened longitudinally and sewn together into one large tube. The latter is transplanted, with intact blood supply, between esophagus and duodenum in place of the stomach. These authors have investigated the ability of four patients so treated to ingest and utilize food. They had been operated on 44, 38, 12, and two months prior to study. Parameters investigated were amount of food tolerated without symptoms, utilization of food as shown by intake and excretion studies and ability to gain weight.

In order to test the first point, the four patients were offered daily for three days a diet consisting of 3,500 calories and composed of 100 g. protein, 100 g. fat and 550 g. carbohydrate. They were asked to eat as much as they wanted but not to force themselves to eat. Ten healthy medical students were offered an identical diet as controls. All patients had good appetites and food intake was estimated to be as great as before surgery for all except one. One patient asked for more food than was offered. Symptoms of esophagitis or dumping were not noted. The average daily intake for the four patients as compared with the control group was as follows: protein, 94 and 95 g.; fat, 103 and 96 g.; carbohydrate, 510 and 527 g.; and calories, 3,345 and 3,349. Thus, the average daily intake was practically the same for the patients with the jejunal pouch as for the control group. This fact indicates that this prosthetic stomach allows the intake of an adequate quantity of food containing protein, fat, and carbohydrate in optimum ratios.

To assess the ability of the patients to assimilate food, protein and fat intake and excretion studies were done. Carmine was given as a marker at beginning and end of a three day test period and all stools passed between these markers were collected and analyzed for protein (kjeldahl) and fat (J. H. van de Kamer, H. ten Bokkel Huinink, and H. A. Weyers, *J. Biol. Chem.* **177,** *347 (1949)*). Intake was carefully catalogued. Medical students were again used as controls. During this period the four patients ingested 94 g. protein and excreted 17 g., retaining 81 per cent, while the controls ingested 95 g. and excreted 11 g., retaining 87 per cent. At the same time the patients with total gastrectomy ingested 103 g. of fat and excreted 16 g., retaining 85 per cent, while the controls ingested 96 g. and excreted 5 g., retaining 91 per cent. Here again there seemed to be little difference between control and treated groups. The ability to absorb protein and fat was relatively normal. Carbohydrate utilization was considered adequate, since patients exhibited neither diarrhea nor glucosuria.

All four patients gained weight on the dietary regimen. From a postoperative low weight, gains ranged from 26 to 33 pounds and it was estimated that all attained from 86 to 98 per cent of what was considered to be the normal weight before illness.

These results agree with previous studies concerning the efficacy of a jejunal pouch in place of the removed stomach (A. H. Moreno, *Ann. Surg.* **144,** *779 (1956)*). These observations, though limited in scope, indicate that fat, carbohydrate, and protein are normally absorbed and utilized when an artificial reservoir is used in place of the stomach. This observation is

extremely important. Previous studies have stressed abnormalities in absorption in gastrectomized patients, particularly in regard to fat, protein, iron, and vitamin B_{12} (*Nutrition Reviews* **15,** *171, 302 (1957)*; **16,** *9, 307 (1958)*; **17,** *7, 297 (1959)*). The improved absorption and utilization of fat and protein in the patients under study probably resulted from several factors. The first is simply the fact that the food intake was normal in amount. The authors stress the point that fear of eating was alleviated since the untoward symptoms from esophagitis and dumping did not occur. They conclude that much of the nutritional debility after total gastrectomy may ultimately be due to decreased food intake due to fear.

Another factor which probably accounted for a comparatively good nutritional status in these patients was the fact that a physiologic reconnection of the alimentary tract was made. Either of two broad types of reconnection of the upper gastrointestinal tract are commonly made after gastric resection. In one, continuity is re-established by anastomosing the residual portion of stomach or esophagus to the duodenum, while in the other an anastomosis of the stomach or esophagus is made with the jejunum and a blind loop of duodenum is left. Food does not pass through this blind loop but some very important digestive promotors are emptied into it (*i.e.* pancreatic juice and bile). It has been frequently observed that absorption of fat is poorer in those patients with the latter type of anastomosis (*Nutrition Reviews* **15,** *171 (1957)*; **16,** *9 (1958)*). The patients in the study reviewed had the first type anastomosis and gained the advantages which accompany this type. A third factor which affects absorption is the speed of passage of food through the intestinal tract. It may be that the pouch, by serving as a reservoir, allows a slower entrance of food into the duodenum, thus to some extent controlling the speed of passage. If the beneficial results of this technique are confirmed in a larger number of patients it should enjoy frequent use. This study suggests that it is the procedure of choice from a nutritional standpoint.

Of interest, but not investigated in this study, would be the effect of the procedure on vitamin and mineral absorption, particularly of iron and B_{12}, since these substances are frequently poorly absorbed in gastrectomized patients (*Nutrition Reviews* **16,** *307 (1958)*; **17,** *7, 297 (1959)*).

ZINC REQUIREMENTS OF POULTRY

The zinc requirement of turkey poults for normal growth, feathering, and bone development appears to be 70 p.p.m. in a diet which contains protein from soybean oil meal. Chickens have a somewhat lower requirement, approximately 35 p.p.m. Zinc chloride, zinc sulfate heptahydrate, and zinc carbonate appear to be readily available sources of zinc for poultry.

A number of experiments during the past few years have demonstrated that dietary zinc is essential for turkey poults to achieve normal growth and bone and feather development. Many of these studies have been carried out with synthetic diets and have indicated that approximately 60 to 65 p.p.m. of zinc are required for optimum development of the turkey poult (F. H. Kratzer, P. Bohra, J. B. Allred, and P. N. Davis, *Proc. Soc. Exp. Biol. Med.* **98,** *205 (1958)*). Most practical turkey rations will not contain this much zinc unless zinc supplements are included. Consequently, it is interesting to study the effects of zinc supplements used with practical poultry

rations and to observe the various factors which may increase the zinc supplement requirement.

W. C. Supplee, R. D. Creek, G. F. Combs, and D. L. Blamberg (*Poultry Sci.* **40,** *171 (1961)*) have reported on the supplementation of a practical poult ration with zinc in the form of zinc chloride. The results of these investigators indicate that the practical diet did not contain sufficient zinc to meet the needs of a rapidly developing poult. Approximately 50 p.p.m. of zinc in the diet was required to support maximum growth and approximately 70 p.p.m. was required for optimum bone and feather development. On the basis of their work, they suggest that at least 30 p.p.m. of supplementary zinc should be added to starting rations for this species. As the birds mature the level of zinc required in the feed is appreciably reduced and very likely is related to the rate of growth.

On a corn, soybean meal type ration which contained 42 p.p.m. of zinc, 96 per cent of the poults showed abnormal feathers at four weeks of age. On the other hand, the abnormal hocks which were present in over 25 per cent of the birds at two weeks of age had almost completely disappeared by four weeks of age on this level of zinc.

Increasing the level of zinc in the diet to 63 p.p.m. had overcome the depressing effect on growth of a zinc deficiency, but there was still some incidence of hock disorder and feather abnormality in these birds.

While Supplee, Creek, Combs, and Blamberg carried out their experiments with Medium White turkey poults, T. W. Sullivan (*Poultry Sci.* **40,** *334 (1961)*) carried out experiments with Broad Breasted Bronze turkeys and obtained results which were similar. Sullivan paid particular attention to the level of calcium and phosphorus in the diet and included 1.9 per cent calcium and 0.95 per cent phosphorus. He used a semi-synthetic diet based on sucrose and isolated soybean protein. His basal diet contained 14 p.p.m. of zinc. The extreme zinc deficiency which occurred on this diet was characterized by poor growth, extremely poor feathering, and the development of very abnormal bones. With each increase in dietary zinc level up to and including 44 p.p.m., there was a significant increase in bone length in these turkey poults. Beyond this level, however, there were no significant increases in the length of the long bones, although the bones of poults receiving 64 p.p.m. of zinc were the longest. Interestingly enough bones from poults receiving 14 p.p.m. of zinc, although shorter, contained a significantly higher percentage of ash than bones from poults receiving levels of zinc of 44 p.p.m. and above.

Another characteristic of the poults fed the low zinc diets was the swollen hock condition which has been related to zinc deficiency by other investigators. Sullivan concludes, on the basis of his investigations, that 50 p.p.m. of dietary zinc will permit maximum weight gain, but that 70 p.p.m. of dietary zinc are required for rapid and normal bone and feather development. These figures agree with those of Supplee, Creek, Combs, and Blamberg.

As a part of his study of the zinc requirement of poults, Sullivan investigated the availability of zinc from different zinc compounds (*Poultry Sci.* **40,** *340 (1961)*). He studied the availability of zinc from zinc sulphate (heptahydrate), zinc chloride and zinc carbonate, and found no significant differences in availability from these three compounds. On the other hand, the amount of zinc from these three compounds was significantly greater than that from zinc oxide or zinc sulphate (monohydrate). Sullivan does not suggest the reason for the difference in availability of zinc from the different compounds, although he does point out that the hygroscopic nature of zinc chloride would limit its use in poultry rations. His results with zinc oxide differ considerably from those reported by H. M.

Edwards, Jr. (*J. Nutrition* **69,** *306* (*1959*)), and taken together the work of Sullivan and the work of Edwards suggest that factors other than the identity of a compound may influence its utilization by turkey poults.

The zinc requirement of chickens has also come under study, since this element is known to be required for hatchability and development. The influence of both calcium and soybean protein upon zinc requirements of young chicks has been reported (R. H. Robertson and P. T. Schaible, *Science* **127,** *875* (*1958*)). M. M. Rahman *et al.* (*Poultry Sci.* **40,** *195* (*1961*)) have reported on the role of zinc in the development of pullets. These investigators used a basal diet of the glucose, purified soybean-protein type and followed the development of birds beginning at one day of age. Their basal diet contained 14 p.p.m. of zinc.

The symptoms of zinc deficiency which were observed were striking. By three weeks of age there was a marked difference between the weight and size of the birds on the deficient diet and those receiving a zinc supplement. The deficient birds showed leg weakness and were inactive or stood on their hocks. They had an unsteady gait by the fifth week and by the sixth week would not move unless disturbed. The hock joints were characteristically enlarged. They had poor feathering, brittle feathers, and unlaced, thorny barbules on the feathers. Feathering rate was very poor and the juvenile feathers remained on the head and neck of these birds. The fact that zinc is a structural component of the feather may in part explain these changes. The skin on the back and neck was scaly, with some indication of a keratosis. The deficient birds had a high respiration rate.

In chickens, as in turkeys, the femurs of the deficient birds become shortened and thickened, as do the other long bones. These workers did not find the increase in ash percentage which has been observed by others. While the bones weighed less than bones from the supplemented birds, in terms of hundred grams of body weight, the deficient birds had a higher percentage of bone. Rahman *et al.* noted that the protein content of the serum of the deficient birds was only 81 per cent of that of the normal controls, but that the albumin-globulin ratio remains constant. The packed cell volume, the cell count, and the hemoglobin content of the blood were all increased by zinc deficiency, but these changes were probably due to hemoconcentration rather than to changes in the erythrocytes themselves. The birds which were unsupplemented required 38 per cent more time to reach sexual maturity than did the supplemented birds. On this diet the addition of 20 p.p.m. of zinc, either in the form of zinc acetate or in the form of zinc bacitracin, was completely effective in eliminating the changes due to zinc deficiency.

Still another effect of zinc has been reported for laying hens by C. H. Whiteside, T. M. Ferguson, B. L. Reid, and J. R. Couch (*Poultry Sci.* **40,** *313* (*1961*)). These investigators, searching for the effect of unidentified factors on egg production, were using a yellow corn, milo, soybean oil meal type ration which they supplemented with such unidentified factor sources as condensed fish solubles, dried whole whey, and zinc and molybdenum. Since the unidentified factors and the combination of molybdenum and zinc resulted in improved egg production and in increased egg weight, it is interesting to speculate that some of the response from the unidentified factor sources may have been due to the trace mineral content. This possibility is especially pertinent in view of the report of Supplee, Creek, Combs, and Blamberg noting that the growth response to supplemental zinc was appreciably greater in the absence of these supplements, and conversely the responses to these supplements were correspondingly less in the presence of added zinc. The benefit from the added zinc and molybdenum also included improved feed utilization.

The evidence is quite conclusive that a deficiency of zinc in poultry diets results in abnormal feathering and changes in the shape and composition of the bones. The zinc requirement appears to be somewhat related to the rate of growth. The turkey poult, with its extremely rapid growth rate, has a requirement of approximately 70 p.p.m., whereas a level of 35 p.p.m. may be adequate for the growing chick, depending somewhat upon the dietary components. The fact that the practical poultry rations may not contain amounts of zinc adequate to meet the requirements of these rapidly growing birds emphasizes the necessity of adding supplemental zinc. Since zinc compounds vary considerably in their availability to young birds, selection of the compounds used must be made considering this availability. In spite of the marked effects of a zinc deficiency, a great deal of research remains to be done on the actual function of zinc in birds, and on the identification of compounds in which zinc is an integral part.

NUTRITION, HEALTH, AND LONGEVITY

Rats fed a moderately restricted but adequate diet from weaning until death lived longer and were less subject to disease than controls. Body length and weight were decreased slightly but reproductivity was not impaired.

Although the average life expectancy of a person born in the United States has lengthened progressively from 40 years one hundred years ago to 73 years today, there is reasonable expectation that science can extend the life span somewhat further (W. B. Bean, *Arch. Int. Med.* **104,** *851* (*1959*)). The increase has resulted from many factors, including better housing and sanitation, control of infection, and better diet. In spite of these improvements many persons still die prematurely of degenerative diseases, particularly of the heart and vessels.

Unfortunately, most of our estimates of food requirements are based upon experiments which have regarded growth and development as their end. By this means we have established minimum daily requirements for vitamins, minerals, protein, and calories. By a still less logical premise we have applied the theory that "if a little bit is good more should be better." Accordingly the recommended daily allowances for each nutrient are considerably higher. Whether this philosophy is wise or not remains to be learned. Rapid growth rate has been shown to be *inversely* correlated with survival in rats (*Nutrition Reviews* **16,** *350* (*1958*)).

D. N. Berg and H. S. Simms have studied the survival of rats fed less food than they desired (Berg, *J. Nutrition* **71,** *242* (*1960*) and Berg and Simms, *Ibid*, *255* (*1960*)). They found that the animals seemed healthier and more vigorous at an advanced age if their caloric intake was restricted moderately. They did not drastically underfeed their rats so as to retard growth and sexual development as did C. M. McCay, G. Sperling, and L. L. Barnes (*Arch. Biochem.* **2,** *469* (*1943*)). Unfortunately, the experiments of Berg and Simms were terminated by a fire which destroyed their laboratories when the animals were approximately 800 days of age.

The initial experiment employed a colony of Sprague-Dawley rats which had been maintained under high standards of cleanliness and health. Their diet consisted of vitamin D-free pellets supplying 24.3 per cent crude protein, 4 per cent fat, and 54 per cent carbohydrate. All essential vitamins and minerals were given in adequate amounts. The rats were weaned at 28 days

and equal numbers with the same body weight were selected for each experimental group. Males and females were subdivided into "restricted" and "ad libitum" regimens. Restriction of food was maintained at two levels: rats were permitted to eat either 33 per cent or 46 per cent of the quantities eaten by the ad libitum controls.

The results of the first 800 days of this study indicated that restriction of food prolonged the life of both male and female rats although the females consistently survived longer. Restriction also delayed the onset of the four principal degenerative diseases of rats: chronic nephritis, periarteritis, myocardial degeneration, and muscular dystrophy.

During the fire which destroyed the authors' laboratories, 31 male and 58 female unrestricted rats and 38 male and 17 female restricted rats were destroyed. However, some rats from the 46 per cent restricted group survived. The authors resumed their studies of these 26 males and 20 females to obtain observations beyond the age of 800 days (Berg and Simms, *J. Nutrition* **74,** *23 (1961)*). The data were compared with those derived from a group of 76 female and 89 male ad libitum rats which had lived out their life span prior to the fire. By combining these data the authors found that the body weight of restricted rats averaged 40 per cent less than that of the controls. Tibial length was decreased by only 5 to 8 per cent and body length by only 8 to 13 per cent. These animals, even at 1,000 days of age, resembled healthy young rats. Their fur was glossy, their teeth firm, and their activity lively. By contrast, the unrestricted rats became lethargic and had dull dirty fur and poor teeth. Survival statistics disclosed that restricted males had a life expectancy of 1,005 days compared with 802 days for unrestricted males and restricted females an expectancy of 1,294 days compared with 930 days. Thus, there was a gain of 203 and 364 days survival for males and females respectively.

The lower incidence of disease was equally impressive. Lesions of glomerulonephritis, periarteritis, myocardial degeneration, and muscular dystrophy appeared much later in life and with a lesser frequency in animals fed the restricted diet. Tumor formation also was delayed, although not as markedly.

This study and the previous reports by the authors point out an important concept in modern nutrition. Although data from animal studies cannot be applied directly to clinical situations, the principle of optimal nutrition versus maximal nutrition is gaining acceptance (*Nutrition Reviews* **14,** *349 (1956)*). Certainly the concept that better health can be achieved by somewhat smaller quantities of good food is more attractive to most of us than some of the restrictive and unpalatable diets proposed by a few investigators.

RENAL STONES, MAGNESIUM, AND VITAMIN B_6 IN RATS

When rats are fed diets deficient in pyridoxine, increased dietary magnesium appears to alleviate the deficiency and reduces the incidence of kidney stones.

The work of S. N. Gershoff *et al.* (*Nutrition Reviews* **18,** *284 (1960)*) has shown that a deficiency of vitamin B_6 in cats is associated with an increase in urinary oxalate excretion. The latter frequently leads to the formation of kidney stones. According to A. Hodgkinson (*Proc. Roy. Soc. Med.* **51,** *970 (1958)*), approximately two-thirds of the renal stones in England contain calcium oxalate. For this reason, the relation of dietary pyridoxine and other nutrients to oxalate excretion is of more than theoretical interest.

As early as 1931, it was recognized that

kidney stones could be produced by a dietary deficiency of magnesium. In a study of the effect of mineral imbalances on the growth of tumors, W. Cramer (*Lancet* **2,** *174 (1932)*) found kidney stones in rats that were fed a low magnesium diet.

During six weeks on this diet these rats grew as well as weanling rats receiving 0.63 mg. of added magnesium per 100 g. of diet. The rats on the magnesium-low ration appeared normal except for the kidneys, which were enlarged and had a puckered surface with grey mottling. There was extensive degeneration of the tubules and glomeruli with calcareous deposits in degenerative areas. The rats on the magnesium-low diet showed considerable amounts of albumin in their urine whereas the controls had only traces. About half of the control rats had kidneys which showed slight degenerative lesions some of which contained calcareous deposits. Cramer concluded that "there seems to be yet a second factor—apart from the presence or absence of magnesium in the salt mixture—which in experiments with a synthetic diet can induce degenerative changes although of a different kind and to a much more limited extent. . ." In view of the more recent work in this field, it is possible that the missing factor was pyridoxine, which was not even recognized when the above work was done.

G. Hammarsten (*Lunds Universitets Arrskrit. N.F.* **32,** *12 (1937)*, reported by S. N. Gershoff and S. B. Andrus, *J. Nutrition* **73,** *308 (1961)*) produced calcium oxalate stones in rats fed stock diets which she believed to be deficient in magnesium. This belief was based on the observation that the addition of magnesium prevented the formation of kidney stones. The basal diets used by Hammarsten contained 4 mg. of magnesium per 100 g. of diet. Gershoff and Andrus suggest that the ration used by Hammarsten contained inadequate amounts of vitamin B_6.

To study the relation between magnesium and vitamin B_6 in the production of renal stones, Gershoff and Andrus fed weanling male rats a purified 15 per cent casein diet. This ration was complete in all respects except for the omission of pyridoxine and magnesium.

The six rats that had been maintained on a diet free of pyridoxine and supplemented with 20 mg. of magnesium per 100 g. of diet showed a weight gain of 11 g. during the six week experimental period. Supplementation of 100 g. of this diet with 400 mg. of magnesium produced a weight gain of 24 g. during the six week period. The latter magnesium supplement also increased the citric acid excretion from 4.2 mg. per 24 hours to 29.6 mg. for the low and high groups respectively. On the pyridoxine deficient diet, magnesium had no effect on oxalate excretion. Magnesium did, however, decrease the xanthurenic acid excretion from 1.59 to 0.76 mg. per 24 hours.

With an adequate level of vitamin B_6 (0.4 mg. per 100 g.), dietary levels of magnesium of 20 or 40 mg. per 100 g. had no effect on any of the parameters mentioned above. This was also true for the high level of magnesium (400 mg.) except for the six week weight gain, which in this case was reduced from 175 g. and 183 g. for the low and adequate magnesium levels respectively to 133 g. for the high level. The reduced weight gain was probably due to the toxic effects of the high level of magnesium.

At the end of the six week feeding period, all rats were sacrificed and their kidneys examined for the presence of stones. Calcium oxalate deposits were seen only in the rats receiving no pyridoxine. In these animals, there was an inverse relationship between the level of dietary magnesium and the extent of calcium oxalate deposition. When the kidneys of the rats receiving the low and high magnesium levels were compared, the inverse relationship was especially prominent for papillary apical incrustations and their intratubular oxalate deposition. The kidneys of the rats receiving the lowest level of magnesium frequently showed large amounts

of oxalate in the nephron. This phenomenon was rarely seen, except in trace amounts, among the rats receiving 40 mg. of magnesium per 100 g. of diet.

In contrast to the oxalate deposits, apatite (calcium phosphate) crystals were seen almost exclusively in those groups receiving 4 mg. vitamin B_6 per 100 g. of diet. Again, there was an inverse relationship between the magnesium level of the diet and the number of animals showing apatite deposits.

These studies indicate an intimate relation between the magnesium level of the diet and the pyridoxine requirement of the rat. They suggest that, in so far as growth and urinary excretion of citrates, oxalates, and xanthurenic acid are concerned, high levels of magnesium appear to have a sparing effect for very low dietary levels of pyridoxine. Just how this effect occurs is unknown. It is recognized that magnesium is essential for many enzyme reactions associated with metabolism. It may be that some of the vitamin B_6-dependent enzymes are especially sensitive to magnesium. This suggestion of Gershoff and Andrus however, requires experimental verification.

These studies emphasize the fact that the level of magnesium used in most purified rations (40 mg. per 100 g.) is probably on the borderline of adequacy. This fact may be especially true if the pyridoxine level of the diet is reduced or perhaps if the calcium content is increased.

COMPOSITION OF MILK

In a new report on the composition of human and cows' milk, variations in yield and composition are stressed. Tables listing average values are included.

It is common practice among clinicians to assume that milk is a fluid of rather constant composition. Calculations are frequently made as to what quantity of protein, calories or some other constituent is consumed by a baby from the volume of milk ingested. To a lesser extent nutritionists have followed the same practice, for there are a number of studies in the literature in which the results of metabolic balance investigations are based on *assumed* values for milk content rather than measured values. In this age of standardization of so many consumer items, it is only too easy to tacitly conceive of milk as a "standard" product.

The recent report of the Committee on Nutrition of the American Academy of Pediatrics (*Pediatrics* **26,** *1039 (1960)*) is therefore timely and informative. The Committee reviewed previous work on the composition of milk, such as the report of Macy *et al.* (*National Academy of Science–National Research Council, Pub. 254, Washington, D.C. (1953)*) and has added new information of importance. Only data on cows' milk and human milk are included in this report. Variations in yield and composition are stressed, as well as average values.

The common practice of pooling milk from many cows in a herd, or even from several herds, prior to marketing the milk greatly reduces the variability of this product. However, there are seasonal variations. For example, protein content varies from a low of 3.2 g. per 100 ml. in March and April to a high of 3.6 g. per 100 ml. in October and November. Phosphorus content is highest (0.108 g. per 100 ml.) in May and in December, reaching a low of 0.098 g. per 100 ml. in August. On a yearly basis, the coefficients of variation for protein, ash, Ca, and P are about 3 per cent. Wider variations are found among the fatty acid constituents. For example, linoleic acid comprises 2.1 $\pm$ 0.7 per cent (standard deviation) of total fatty acids, oleic acid

17.7 ± 4.6 per cent, and linolenic acid 1.7 ± 0.7 per cent. The values for fatty acids were obtained by gas chromatography.

Variations are much more pronounced in the case of human milk, the variability extending to amount as well as composition. On the tenth postpartum day, for example, the mean volume is about 1,200 ml., with a range of 800 to 1,800 ml.

In assessing the composition of human milk, the report is careful to distinguish between colostrum (one to five days postpartum), transitional (five to ten days), and mature milk.

Studies of mature milk from a large number of women reveal some astounding variations. The coefficient of variation is 18 per cent in the case of lipids, 9 per cent for lactose, 24 per cent for protein, and 13 per cent for total nitrogen. Interesting variations occur during a single nursing, and at different times of the day. There are even variations between the two breasts.

It is interesting that European women produce milk slightly higher in protein (1.4 g. per 100 ml.) than do American women (1.1 g. per 100 ml.). Reference is made to the well-known fact that human milk may reflect to a certain extent the maternal diet. This is particularly true for fatty acids and for vitamin A.

Tables are given which list average values for all of the major constituents of milk, so that human and cows' milk may be readily compared. Included are values for protein, fat, carbohydrate, minerals (including some trace elements), amino acids, lipids, and vitamins. A short discussion of relative biological values of cows' milk and human milk protein, as well as the differences in renal solute load, is included.

Of importance today is the matter of radioactive fission products resulting from fallout from atomic bomb explosions. Values for strontium 90, strontium 88, cesium 137, iodine 131, and barium 140 are given, since these are the most significant fission products to be found in milk. The recent work of S. A. Lough, G. H. Hamada, and C. L. Comar (*Proc. Soc. Exp. Biol. Med.* **104,** *194 (1960)*) on the strontium 90 content of human milk is mentioned. It is of great interest that human milk contains only about one-tenth as much of this isotope as does cows' milk. It remains to be seen whether this difference, though of questionable significance at the biologic level, will be used to promote the practice of breast feeding!

The report concludes with the statement that despite the profusion of data on milk composition, the picture is far from complete. The range of variability of many components, such as minerals and vitamins, is incompletely documented, and the relation between maternal diet and human milk composition insufficiently explored.

The report of the Committee on Nutrition is a valuable and useful compendium of information on milk composition. Its emphasis on the range of variability in this all important food should stimulate further work in this area.

REDUCTION OF SERUM AND LIVER CHOLESTEROL WITH PECTIN

Rats fed a basal diet and cholesterol were compared with similar groups given supplements of pectin. The addition of 2.5, 5 or 10 per cent pectin to the diet reduced concentrations of cholesterol in the blood and liver.

Regardless of inconclusive evidence that hypercholesterolemia actually produces coronary atherosclerosis in man, investigators continue to search for means of controlling the cholesterol content of the blood. M. G. Hardinge and F. J. Stare (*J. Clin.*

Nutrition **2,** *83 (1954)*) observed that the amount of cholesterol in the blood of vegetarians was less than that in the blood of comparable omnivorous persons. Other studies have demonstrated a lesser incidence of coronary heart disease in vegetarians. The factors responsible for these differences may be multiple. They have been attributed by various people to a lower intake of saturated fats, cholesterol or protein. Some believe that the changes are due to a greater intake of cellulose, complex starches or vegetable sitosterols.

Recently A. Keys reported that feeding pectin to patients resulted in a modest decrease in the amount of cholesterol in the blood (*33rd Scientific Session, Am. Heart Assn. (1960)*). Studies in animals have shown that pectin added to the diet apparently reduced the absorption of cholesterol from the intestines (T. M. Lin, S. M. Kim, E. Karvinen, and A. C. Ivy, *Am. J. Physiol.* **188,** *66 (1957)*).

This problem was investigated further by A. F. Wells and B. E. Ershoff (*J. Nutrition* **74,** *87 (1961)*). They selected young male rats and fed them a balanced basal ration which was cholesterol-free. Different groups were given supplements of 1 per cent cholesterol and of various other substances to determine the effect on lipid metabolism. These groups of animals were sacrificed either at four or at six weeks. Blood samples were then collected for measurement of cholesterol and livers were saved for measurement of cholesterol and total lipids.

The first experiment involved seven groups of animals. The first received the basal ration, and subsequent groups were given 1 per cent cholesterol or cholesterol plus 5 per cent pectin, 5 per cent cellulose, 5 per cent agar, 5 per cent sodium alginate or 5 per cent calcium silicate. Those animals receiving only the cholesterol supplement had a significant rise in hepatic and serum cholesterol. The total lipid content of the liver increased greatly. However, when 5 per cent pectin was fed along with the cholesterol, these changes were largely reversed. There was no effect from feeding cellulose, agar, sodium alginate or calcium silicate. The addition of pectin to the cholesterol-free diet had no significant effect.

The second experiment compared the effects of feeding either 5 per cent pectin or 5 per cent protopectin to groups of animals eating the cholesterol-free diet or the diet containing 1 per cent cholesterol. It was noted that the pectin largely overcame the effect of the added cholesterol, but had a minimal effect in those animals fed the basal ration. However, protopectin lacked any effect.

In the third study, the authors compared the effects of feeding graded levels of pectin or pectin derivatives. Again, the rats were fed either the basal ration or this ration plus 1 per cent of cholesterol. The various groups received supplements as follows: 2.5 per cent citrus pectin, 5 per cent citrus pectin, 2.5 per cent apple pectin, 5 per cent apple pectin, 5 per cent pectic acid, 5 per cent polygalacturonic acid, 5 per cent galacturonic acid, 5 per cent gastric mucin, 10 per cent gastric mucin, 15 per cent protopectin, 1 per cent sulfasuxidine plus 0.05 per cent streptomycin, and 1 per cent sulfasuxidine plus 0.05 per cent streptomycin plus 5 per cent citrus pectin. These animals were found to have concentrations of cholesterol in the blood and liver which varied with the amount of pectin fed. Thus, the 2.5 per cent level of pectin exerted a definite depressant effect and the 5 per cent level a greater effect. In a footnote the authors stated that additional studies showed a still greater effect when 10 per cent pectin was fed. The effects of citrus or of apple pectins seemed to be identical. However, pectic acid, polygalacturonic acid, and galacturonic acid had no significant effects. Similarly, gastric mucin and the antibiotics were ineffective. The addition of antibiotics to the regimen of a pectin fed group did not augment the effect of pectin significantly.

In the fourth and final experiment the authors studied the effect of feeding cholesterol on one day and pectin on the next for a period of four weeks. They did the same with a group of animals given cholesterol-free diets. The same cholesterol and lipid lowering effects were noted in the group with cholesterol in the diet, but no effect was noted in the other group.

The authors mentioned that preliminary measurements of fecal cholesterol failed to indicate an increased rate of excretion as a result of feeding pectin. The fact that feeding cholesterol and pectin on alternate days was effective suggests that a physical complex between cholesterol and pectin or impaired absorption across the intestinal mucosa probably does not account for all the effects noted. There was no evidence that pectin exerted its effect by altering the intestinal flora since the addition of antibiotics failed to simulate or augment the effect.

This study adds new information regarding the absorption of cholesterol and lipids from the gastrointestinal tract. This is a phase of lipid metabolism which has lagged behind other investigations chiefly because of the difficulties engendered by the action of intestinal bacteria. However, this study seems to be a significant start and it is hoped that much more information will soon become available. Naturally, one cannot advise indiscriminate administration of pectin to patients until much more is learned.

NUTRITION, TOOTH SIZE, AND CARIES SUSCEPTIBILITY

A protein deficient diet throughout pregnancy and lactation in rats resulted in a reduction in the size of the molar teeth of the offspring, an altered cuspal pattern, retarded eruption of the third molars, and an increase in caries-susceptibility.

The genetic constitution of an animal provides a blueprint in very specific detail for the characteristics of tooth size, shape, and caries-susceptibility. However, the full implementation of this blueprint may not be possible if nutritional demands during tooth development are not adequately met. The first clear experimental evidence of this fact in the rat has been reviewed earlier (*Nutrition Reviews* **15,** *213 (1957)*). In these early studies, the offspring of female rats which were fed a vitamin A deficient diet, a diet supplemented with a low level of sodium fluoride or a diet with a low calcium-phosphorus ratio during pregnancy and lactation, had smaller teeth than did the controls. In addition, the offspring of the deficient females had teeth whose cuspal pattern had been altered appreciably by the dietary manipulations. The results of the three regimens were similar, suggesting a nonspecific type of mediating influence.

The molars of rats from caries-susceptible and caries-resistant strains are characteristically different from each other. It has been reported both for the Hunt-Hoppert caries-resistant and caries-susceptible strains and for the Harvard caries-resistant and caries-susceptible strains of rats that there are significant differences in the size, shape, and cuspal patterns from strain to strain (P. F. Kifer, H. R. Hunt, C. A. Hoppert, and C. J. Witkop, *J. Dent. Res.* **35,** *620 (1956)* and R. M. Grainger, K. J. Paynter, and J. H. Shaw, *Ibid.* **38,** *105 (1959)*). While these characteristics have high correlations with caries-resistance, it is not at present known whether these correlations have strict positive relationships to the susceptibility to tooth decay. However, it is clear from these studies that the genetic constitution of these animals clearly determines the physical characteristics of the teeth.

The interrelationship of the hereditary

and infectious elements of experimental tooth decay have been reviewed earlier (*Nutrition Reviews* **19,** *105 (1961)*). In a further exploration of the influence of heredity, a crossbreeding experiment was conducted with the Harvard caries-resistant and caries-susceptible strains of rats (J. H. Shaw and D. Griffiths, *Arch. Oral Biol. (London)* **3,** *247 (1961)*). The same adults of the two strains with appropriately alternated matings were used for the production of pre-experimental and post-experimental purebred controls and of experimental crossbreds. The dental caries incidence of the individual offspring covered a very broad and continuous spectrum ranging from high caries-susceptibility to high caries-resistance. The average caries experience of the offspring of the cross of resistant males and susceptible females was almost identical to the average for the opposite cross of susceptible males and resistant females. The average dental caries experience of these two populations of crossbred rats was significantly higher than that of the average of the purebred rats of the resistant strain and significantly lower than that of the average of the purebred rats of the susceptible strain. The male and female parents exerted about equal influences upon the caries activity of the offspring, while the nursing mother had relatively little if any influence upon the caries incidence of fostered offspring.

In a recent study, P. J. Holloway, J. H. Shaw, and E. A. Sweeney (*Arch. Oral Biol.* **3,** *185 (1961)*) observed the effect of purified diets containing various sucrose-casein ratios on tooth size and shape, time of tooth eruption, and caries-susceptibility of offspring of females from the Harvard caries-susceptible strain. Their main study was composed of three groups.

In the first group there were 15 females which served as controls and were fed a purified diet containing 24 per cent casein. In the second group were 15 females which were fed a high protein diet containing 72 per cent casein, while 32 rats in the third group were fed a low protein diet containing 8 per cent casein. The diets for the second and third groups were prepared by the inclusion of the high and low amounts of protein at the expense of sucrose isocalorically. The diets were otherwise adequate in vitamins, unsaturated fatty acids, and salts. The females were all maintained on the diets for a preliminary period of 28 days, after which they were mated with sibling males and continued on their respective diets throughout pregnancy and lactation.

Within three days after parturition, the litters were reduced to six, three males and three females wherever possible. The offspring were weaned when they were 25 days of age. The rats in the first group were continued on the normal protein diet until 75 days of age, at which time they were sacrificed.

In groups two and three, one rat was selected at random from each litter at weaning and transferred to the normal protein diet, while the remainder of the offspring in groups two and three were continued on the high protein and low protein diets, respectively, until sacrifice at 75 days of age. Forty-six of the females were mated again 110 days after the beginning of the experiment, 24 on a normal protein diet and 11 each on the high protein and low protein diets. The litters in this second series were not reduced in number and were divided into approximately equal halves at weaning. One-half of the offspring was continued on the same diet as the mothers, while the other half was transferred to the normal protein diet, except in the case of the offspring of the normal protein females, where the second half was transferred to the low protein diet. The rats in the second litters were sacrificed at 85 days of age.

The breeding females on the low protein diet showed a reduced rate of growth within the first two weeks of the experiment.

However, the rate of conception among these females was unexpectedly good, although the number of viable offspring in their litters was less than that in the litters of the control females in group one. The pups of the females of the low protein group were much smaller than those of the females on the control diets and their development was retarded with respect to opening of the eyes, development of the external ear, and first movement out of the nest. However, despite their small size and slow rate of growth, these offspring were active and reasonably healthy throughout the entire experimental period. Reproduction among the females on the normal and high protein diets was approximately equal, although the rats in the high protein families did not grow quite as well as those on the normal protein diet.

The eruption of both the maxillary and mandibular third molar teeth occurred about five days later in the offspring whose mothers had been fed the low protein diet than in the offspring of the control females or of the females in the high protein group. It is interesting to note that the third molars of the offspring in the high protein group did not have a detectably different eruption time from that of the controls, despite the fact that they had grown much more slowly than the animals from the control females. On the first visual examination of the teeth of the protein deficient offspring, it was obvious that there was an appreciable reduction in the size of the teeth from that of the controls. These reductions were found to be significant and varied from a 3.2 per cent reduction in the bucco-lingual width of the first maxillary molar to a 15.8 per cent reduction in the mesio-distal length of the third mandibular molar.

Differences in the shape of the teeth were also observed. Some of the subsidiary cusps of the teeth of the offspring in the low protein group were affected, particularly on the third molars. Some cusps were obviously reduced in size and others were missing altogether. This was particularly noticeable with respect to the small antero-buccal cusps of the lower third molar. Among the 58 offspring whose mothers had received the low protein diet and which had been continued on the low protein diet until the end of the experimental period, 40 had one or more third molar teeth with missing cusps. It is obvious from these data that the influence of the low protein diet had been entirely mediated before weaning, since of the 58 offspring whose mothers had received the low protein diet but which had been transferred to the normal protein diet at 25 days of age, 41 of these had third molar teeth with one or more missing cusps.

The animals whose mothers had been maintained on the low protein diet throughout pregnancy and lactation had caries indices which were much higher than those for the animals whose mothers had been fed the diet with the normal protein level. This difference was highly significant for both series of litters and for each individual comparison. The offspring of mothers receiving the high protein diet during pregnancy and lactation had slightly less tooth decay than the offspring of the females on the normal protein diet. This difference again was significant, but the actual difference was much lower than the increase observed in the protein deficient animals. Here again the influence was largely mediated during pregnancy and lactation, for the difference between littermates where the diet had been changed at weaning was not as great as the difference between total groups.

In this study, it was obvious that the dietary regimen imposed upon the females during pregnancy and lactation had significant influences upon the size, the morphology, the caries-susceptibility, and the time of eruption of the molars. One should not conclude that these nutritional influences upon the maternal organisms produced by the isocaloric substitution of sucrose for casein were directly related to

protein deficiency. Inanition may have played an important part, as there was some evidence that the females on the low protein diet were not providing an adequate amount of milk of adequate quality. Phosphate content also varied among these three diets and may be an additional factor to have influenced the end results. Thus, on the basis of this experiment one is unable to define the exact cause of the observed effects. However, this inability is less important than the over-all observation of the striking changes that can be forced upon the teeth as a result of dietary aberrations during tooth development, with the obvious sequelae that the normal blueprint laid down by the genetic constitution of the strain had not been realized.

FATTY ACID CHAIN LENGTH AND METABOLIC RATE

Four different triglycerides containing C^{14}-labeled fatty acids of different chain lengths were fed and the rates of metabolism determined in rats.

The rate of oxidation of fatty acids to CO_2 is influenced by the nutritional state of the animal, the route of administration, and the form of the fatty acid. The effect of chain length on the rate of metabolism of C^{14} carboxyl labeled fatty acids is reported by S. L. Kirschner and R. S. Harris (*J. Nutrition* **73,** *397 (1961)*).

Tagged butyric (C_4), caprylic (C_8), lauric (C_{12}) or palmitic (C_{16}) were fed rats by stomach tube in the form of randomly mixed synthetic triglycerides. Preparation was accomplished by mixing glycerol with the suitable C^{14}-labeled acyl chloride in the presence of pyridine. Unreacted acyl chloride was removed by alkaline wash. Larger amounts of unlabeled C_4, C_8, C_{12}, and C_{16} triglycerides were prepared for diluting the radioactive triglycerides. Using benzyl trimethyl ammonium hydroxide as a catalyst, methyl esters were reacted with glycerol in vacuo at 100°C. Tributyrin was purified by distillation, tricaprylin by deodorization, and trilaurin and tripalmitin by crystallization.

The only difference among the various triglyceride mixtures was the presence of tagged C_4 acids in one and tagged C_8 acids in another and so forth. The random arrangement was achieved by combining safflower oil with the triglycerides in the presence of a sodium methoxide catalyst agitated at 70–80°C. for 45 minutes. After neutralizing the catalyst the samples were dissolved in ether, washed, dried, and deodorized. Unlabeled mixed triglycerides were added to the C^{14}-labeled blends.

Male albino rats weighing about 160 g. were placed under mild ether anaesthesia and given 0.8 ml. of labeled triglyceride by stomach tube after an overnight fast. Using a metabolism cage ventilated with CO_2-free air, the expired samples were collected in 4N sodium hydroxide. At intervals the CO_2 was precipitated as $BaCO_3$ and counted using a thin window flow counter. The diet fed during the 48 hour collection period included 30 per cent casein, 15 per cent untagged fat, and about 50 per cent corn starch. Feces and urine collections were analyzed using a combustion technique producing CO_2 which was then precipitated and counted as before. Four rats were fed the palmitic 1-C^{14} acid glyceride and three rats each were given one of the other labeled triglycerides.

The average per cent of radioactivity recovered from the colon and feces after 48 hours was butyric, 0.5 per cent; caprylic, 0.6 per cent; lauric, 1.8 per cent; and palmitic, 13.5 per cent.

The percentage of absorbed radioactivity recovered from the breath of rats was calculated during each collection period. During the first four hours of the collection

the rates of excretion of radioactivity were significantly higher after administration of butyric or caprylic 1-C^{14} acids than of the longer-chain acids.

Although the $C^{14}O_2$ excretion rate of the lauric acid group was highest during the 8 to 12 hour period the values were not significantly different from group to group. During the 12 to 16 hour period the rate for the lauric acid group was significantly greater than for butyric or caprylic but not palmitic. Subsequent rates were not significantly different (16 to 24 or 24 to 48 hours). During the 48 hours, butyric and caprylic acids were metabolized at about the same rate. The maximum hourly respiration rate of butyric acid was 17.0 per cent during the one to two hour period. For caprylic acid the maximum was 17.7 per cent, also during the one to two hour period. The maximum for lauric acid was 7.0 per cent during the two to four hour period; for palmitic acid it was 4.8 per cent during the four to eight hour period.

Since absorption of the fatty acids was completed in a four to eight hour period they were either deposited in the tissues or metabolized in the 12 hours after feeding. Activity recovered from the breath after 16 hours apparently was first deposited, then mobilized and degraded.

Shorter-chain fatty acids (C_{10} and C_{12}) or their triglycerides (fed in corn oil) are absorbed directly into the blood stream, whereas long-chain acids (C_{14}, C_{15}, C_{16}, and C_{18}) are absorbed almost entirely in the lymph, according to the report of B. Bloom, I. L. Chaikoff, and W. O. Reinhardt (*Am. J. Physiol.* **166,** *451 (1951)*) and Bloom *et al.* (*J. Biol. Chem.* **184,** *1 (1950)*). In the present experiment the rapid transport of shorter-chain fatty acids in the portal system to the liver could account partly for their more rapid metabolism. On the basis of the experiments of R. G. Gould *et al.* (*Ibid.* **177,** *295 (1949)*) which demonstrated that all carbons of a fatty acid molecule are converted to CO_2 at about the same time, it would be expected that complete conversion of a labeled fatty acid would be represented by the $C^{14}O_2$ following butyric or caprylic 1-C^{14} acids rather than either lauric or palmitic 1-C^{14}. Analysis of urine radioactivity shows little difference among groups. The results indicate that most of the fatty acids and their products are metabolized to $C^{14}O_2$ or deposited in the tissues with little excretion in the urine.

IMPAIRED ANTIBODY PRODUCTION IN PYRIDOXINE DEFICIENT GUINEA PIGS

Pyridoxine deficient guinea pigs had impaired antibody responses to diphtheria toxoid. Allergic responses to diphtheria toxin were also reduced.

The inability of pyridoxine deficient animals to form antibodies has been reviewed previously (*Nutrition Reviews* **10,** *353 (1950)*; **15,** *47 (1957)*; **19,** *116 (1961)*). A. E. Axelrod and his group have studied rats deficient in pyridoxine or other vitamins and reported that antibody responses to human erythrocytes, to diphtheria toxoid, and to influenza PR-8 antigen were inhibited.

In order to learn whether pyridoxine deficiency had the same effect in other species, Axelrod and his group performed similar studies with a group of guinea pigs. In addition to the circulating antibodies, they studied skin reactivity of immunized animals to diphtheria toxin (A. E. Axelrod, S. Hopper, and D. A. Long, *J. Nutrition* **74,** *58 (1961)*). They selected young male guinea pigs and fed them either a purified diet which could be supplemented with known quantities of pyridoxine or a standard

laboratory ration. In these studies the animals were not pair-fed. Pyridoxine deficiency was induced either spontaneously by feeding a deficient diet or by daily administration of desoxypyridoxine hydrochloride 10 mg. per 100 g. of body weight intraperitoneally.

In their first experiment they used four groups of animals: a control group fed a laboratory ration, a second group fed a purified diet supplemented with 16 mg. of pyridoxine hydrochloride per kg. of diet for eight weeks, a third group fed this diet for four weeks after which time pyridoxine was removed from the diet, and a fourth group fed the pyridoxine deficient diet for the entire eight week period. The fourth group died prior to the termination of the study.

After four weeks all of the animals were immunized intraperitoneally with 7.5 Lf units of diphtheria toxoid. Three weeks later blood was collected for determination of primary antibody response and a second immunizing dose of diphtheria toxoid was given. After another week blood was drawn for determination of the secondary antibody response. At this time skin tests were performed with diphtheria toxin. The deficient animals gained weight poorly and had unkempt-looking fur, but appeared normal in other respects. Their level of circulating antibodies was materially depressed, especially when compared with the secondary response of the control animals. Their skin reaction to diphtheria toxin as measured by the thickness of a double fold of skin at the site of injection was definitely less than that of the control animals.

The second experiment was performed in a similar manner except that 1) the pyridoxine supplement was reduced from 16 to 4 mg. per kg. of the diet, 2) animals selected for the pyridoxine deficient diet were given injections of pyridoxine for three days prior to the beginning of the experiment, and 3) a smaller dose of antigen was given intramuscularly. The control group received 4 mg. of pyridoxine per kg. of diet throughout the ten weeks of the study. The second group was fed this diet for the first five weeks and a deficient diet for the second five weeks. During the latter half of the study each animal in this group received 10 mg. of desoxypyridoxine hydrochloride per 100 g. of body weight intraperitoneally each day. The third group was fed a deficient diet throughout the study.

At the end of five weeks all animals received 2.5 Lf units of diphtheria toxoid intramuscularly. Blood was collected three weeks after this immunizing injection and a booster dose of antigen was given. A second sample of blood was drawn ten days later and skin tests were applied using diphtheria toxin.

Again the growth curves showed that pyridoxine deficient animals gained weight poorly. Their production of circulating antibodies was inhibited sharply in both the primary and secondary phases. Skin reactions to diphtheria toxoid, as estimated by the thickness of a fold, was less marked in the deficient than in the control animals.

The authors concluded that pyridoxine deficiency can be induced in guinea pigs either by feeding a deficient diet or by administering the pyridoxine-antagonist desoxypyridoxine to animals previously fed an adequate diet. They noted, however, that the guinea pig was more resistant to desoxypyridoxine than was the rat. Although there was a relative paucity of signs of pyridoxine deficiency, the impairment of antibody production was similar to that observed in rats. The authors commented upon the fact that their animals were not pair-fed. They contended that previous experiments have demonstrated normal antibody production in undernourished rats. Nevertheless, it would seem desirable to have controlled this study more precisely by pair-feeding.

Studies by L. J. Wayne, *et al.* (*Arch. Int. Med.* **101,** *143 (1958)*) indicated that pyridoxine deficiency in man did not inhibit

antibody production. However, a combined deficiency of pyridoxine and pantothenic acid in man has been reported to impair antibody production sharply (R. E. Hodges and W. B. Bean, *Am. J. Clin. Nutrition* **9,** *244 (1961)*).

Certain infectious diseases are vitamin dependent. This has led to speculation that an allergic factor is involved in producing some of the manifestations (H. A. Schneider, J. M. Lee, and P. K. Olitsky, *J. Exp. Med.* **105,** *319 (1957)*). Other applications of vitamin deficient states are suggested by the studies of skin graft survival. Pyridoxine deficiency has been shown by several groups to prolong the survival of grafts (A. E. Axelrod *et al.*, *Science* **127,** *1388 (1958)*; B. Fisher *et al.*, *Surgery* **44,** *149 (1958)*; and B. J. Hargis, L. C. Wyman, and S. Malkiel, *Int. Arch. Allergy Applied Immunol.* **16,** *276 (1960)*). A. S. Parkes has demonstrated that ovarian homographs can be protected for a time by inducing pyridoxine deficiency in animals (*Nature* **184,** *699 (1959)*).

These studies indicate that nutritional factors play a vital role in the immunologic response. Further applications of these findings will undoubtedly be of great interest.

ANTIBIOTIC-CONTAINING FOODS AND ANIMAL EXPERIMENTATION

Feeding dogs a commercial food preparation containing a small antibiotic supplement (5 g. per ton) reduced the expected development of peritonitis after ligation of the appendix.

For several years many of the commerically available food preparations for experimental animals have been supplemented with certain of the broad-spectrum antibiotics. Antibiotic supplements have been shown to have a growth enhancing effect, although the mechanism of action is not clearly defined (*Nutrition Reviews*, **15,** *348 (1957)*). The importance of the effect of minute amounts of antibiotic contained in food on intestinal bacterial flora is difficult to assess. Some antibiotics work as well or better when administered intravenously (*loc. cit.*).

Recent evidence would suggest that such antibiotic supplements may have rather appreciable effects on bacterial growth in the body and may profoundly alter the outcome of some experimental procedures when included in the diet. I. Cohn, Jr., A. M. Cotlar, and F. S. Massari (*Ann. Surg.* **153,** *585 (1961)*) have reported on the effect of such antibiotic-containing food on the development of peritonitis following ligation of the appendix in dogs. This is a simple, easily performed surgical procedure and commonly results in the development of peritonitis in 60 to 90 per cent of the animals (J. O. Bower, J. C. Burns, and H. A. Mengle, *Arch. Surg.* **37,** *751 (1938)*).

In a preliminary study, the authors achieved only a 10 per cent incidence of peritonitis in dogs similarly treated. Review of the procedures in the authors' laboratory and of those used previously by others led to the conclusion that only the food was remarkably different. In the previous experiments referred to the food had not contained antibiotics, whereas in the authors' experiment the food contained 5 g. tetracycline per ton (Purina Kibbled Meal). This level of antibiotic was confirmed by bioassay with Bacillus mycoides ATCC No. 9634. Although it seemed unlikely that such a small amount of antibiotic (estimated intake of one pound of food per dog per day would contain 2.5 μg) could alter bacterial growth enough to affect the results as previously demonstrated, this seemed to be the only possibility.

In order to test the effect of the antibiotic, the authors fed two groups of dogs on different commercial food preparations for

two weeks and then exposed them to the same experimental procedure. The first group of 14 dogs was fed the antibiotic-supplemented food whereas the second group of 18 dogs was fed a food free of added antibiotics. Concurrently, and under identical conditions, dogs of both groups were subjected to ligation of the appendix. Four of the dogs in group One (28 per cent) developed peritonitis whereas 12 of the dogs in group Two (67 per cent) did so. This difference was statistically significant. Examination of the bacterial flora in animals of both groups disclosed no demonstrable alterations.

The results of this study are of interest to all investigators who use experimental animals fed commercial food preparations. The authors state that the only recognized difference between the regimens employed for the two groups of dogs was the antibiotic supplement. However, they do not give the exact vitamin, mineral or nutrient content of either diet. Since both were commercial preparations one might assume that both were adequate from a dietary standpoint. However, one must always remember that almost any dietary deficiency, even of slight degree, promotes the development of bacterial infections. The authors also state that the bacterial flora were not demonstrably altered. It would be of interest to know what methods they used for study, since the enumeration of intestinal bacteria is laborious and, at best, incomplete.

Although the authors failed to demonstrate any overt change in intestinal microflora, it is likely that the effect of the antibiotic was to alter directly bacterial growth since the development of peritonitis depends on such growth and failure of development means that bacteria did not grow. In certain species it is readily demonstrated that antibiotic-supplemented foods cause changes in the bacterial flora (*Nutrition Reviews*, **14,** *180 (1956)*), while in other species this demonstration is not so easily made (*Ibid.* **15,** *348 (1957)*). However, even in the latter instances it can be demonstrated that antibiotics affect the flora in subtle ways. For instance, certain antibiotics exert vitamin-sparing actions; this effect is not due to improvement of vitamin absorption or utilization by the organism but to increased synthesis by bacterial flora (*Ibid.* **19,** *60 (1961)*). Even though the intake of antibiotic from food supplements was far below the minimal therapeutic doses, it has been demonstrated that this small amount raises the bacterial disease threshold (*Ibid.* **14,** *127 (1956)*).

All investigators who use experimental animals fed foods containing antibiotic supplements should be aware of the possibility of alterations in results due to effects on intestinal flora. This investigation effectively illustrates such an alteration.

NOTES

Vitamin A Toxicity in Whale Liver

It has been recognized for many years that polar bear liver is toxic not only to animals but to man. The toxicity of polar bear liver was shown by K. Rodahl and T. Moore (*Biochem. J.* **37,** *166 (1943)*) to be due to the very high level of vitamin A it contains.

Polar bear liver per gram contains as much as 20,000 I.U. of vitamin A, while the liver of the sperm whale contains 4,400 I.U. In contrast to these high levels, swine and cow liver per gram contain 100 to 150 I.U. (T. Moore, *Biochemistry and Physiology of Nutrition*, ed. by G. H. Bourne and G. W. Kidder, *Vol. 1, p. 269*, Academic Press, New York (1953)).

With the increasing emphasis on the

complete utilization of all food products, it was inevitable that studies should be carried out on the effect of feeding whale liver to fur-bearing animals. D. W. Friend and E. W. Crampton (*J. Nutrition* **73,** *317 (1961)*) fed eight groups of female mink fish rations supplemented with either 5 or 10 per cent whale liver. Each group, consisting of 18 animals, was fed a mixture of codfish fillet waste, a cereal-mineral mixture, and either whole codfish or whale meat (the latter two at a level of 40 per cent). Two different males were put with the females on two successive days in order to assure that the reproductive performance was not influenced by sterility of the males.

In each of the four groups receiving 10 per cent whale liver, fertility was reduced in comparison to the mink receiving the 5 per cent level. The reduction in fertility ranged from as little as 20 per cent to as much as 60 per cent. No indication is given as to whether the whale liver diet, which was presumably started just prior to mating, exerted its effect by preventing fertilization of the ova or had an effect on the fetuses *in utero*.

An interesting auxiliary observation not mentioned by the authors is the fact that the presence of whale meat produced better reproductive performance than did whole codfish. From this standpoint, the whale meat appeared to reduce the toxicity of the liver. For example, only nine out of 18 mink whelped on the 5 per cent whale liver-40 per cent whole codfish ration, whereas 11 out of 18 whelped on the similar ration containing whale meat in place of codfish. In another series, the number of mink that whelped was increased from four in the codfish-10 per cent whale liver ration to eight in the similar ration containing whale meat in place of codfish. A similar improvement in reproductive performance was seen in the other two groups receiving whale meat. The advantage seen when whale meat was fed was consistent and appeared significant.

These studies extend the concept of vitamin A toxicity as seen in polar bear liver to another species of animal. With the introduction of whale meat to the human market, it becomes imperative that precautions be taken to insure that the liver of this species is excluded.

The work of Friend and Crampton should have included a control group receiving a diet recognized as producing the best possible reproductive performance. These studies would have been improved by vitamin A analyses of the rations used.

These investigations should be extended to determine whether differences in the biological value of proteins or their absolute levels in cod and whale can account for the reduction in toxicity of whale liver observed by Friend and Crampton. It would have been helpful if their report had indicated the changes in body weight experienced by the animals on the different diets. Such data might have indicated whether protein was involved in the phenomenon reported. Since the literature provides no information on this point, these studies should be extended to incorporate the influence of proteins on vitamin A toxicity.

Liver Regeneration After Partial Hepatectomy in Cirrhotic Rats

Normal liver has the capacity to regenerate. In a normal rat the liver returns to its original weight within two weeks after a partial hepatectomy. On regeneration the structure and functional capacity of the newly formed liver apparently is similar to that of the normal liver. Following the destruction of one lobe of the liver in human beings or dogs, there is a compensatory increase in size of the remaining lobes.

Liver damage can be produced in numerous ways in animals and man, as with toxic substances such as carbon tetrachloride or through poor nutrition (*e.g.* low protein, high fat diets). While the atrophic cirrhotic liver is not usually believed to show regenerative capacity following a partial

hepatectomy, N. Rabinovici and E. Wiener (*Gastroenterology* **40**, *416* (*1961*)) recently restudied the effect of partial hepatectomy in rats which had carbon tetrachloride-induced cirrhosis.

They produced cirrhosis in a large number of white male rats weighing 200 to 300 g. by allowing them to inhale carbon tetrachloride twice a week for six weeks. Half of the animals thus treated did not develop macroscopically detectable cirrhosis. For this study only animals with cirrhosis of sufficient severity, indicated by a coarse, hard liver of the atrophic or hypotrophic type with or without ascites, were used. About one-half of the animals which developed cirrhosis were in this category.

Two weeks to two months after the carbon tetrachloride inhalations were discontinued a subtotal hepatectomy was performed on these animals. The operative mortality rate, both immediately following operation and later, was approximately 25 per cent. Groups of ten cirrhotic animals were sacrificed at various intervals from 12 hours to three months following the operation and the degree of liver regeneration was determined. For controls an equal number of normal animals of the same weight were kept under identical conditions and were subjected to the same type of partial hepatectomy.

These investigators noted that the cirrhotic rat liver had a regenerative capacity similar to that of normal liver. Regeneration of the liver in the cirrhotic animal occurred in two separate growth spurts. The first occurred in the week immediately following the hepatectomy, when there was a high increase in the percentage of regeneration (158 per cent by weight of the operative weight). Between the first and the third week following the operation regeneration slowed down but between the third and the fourth week there was another large increase in growth. While there is speculation concerning the reason for the two separate growth spurts, no definitive cause is given. The amount of liver which regenerated was related to the mass of the residual liver. The smaller amount of residual liver left, the greater was the rate of the liver regeneration. A constant finding in the surviving animals was the disappearance of the ascites following a partial hepatectomy. The author suggests that this may be due either to the increased liver function as a result of the regeneration or to the fact that liver tissue damaged by carbon tetrachloride had been removed. Thus, these investigators have demonstrated that the cirrhotic liver can be regenerated in a manner similar to that of the normal liver.

Recent Books

Food, Nutrition and Diet Therapy. Marie V. Krause. Published by W. B. Saunders Company, Philadelphia and London. Pp. 716. Price $6.75.

Eat Not This Flesh. Frederick J. Simoons. Published by The University of Wisconsin Press, Madison, Wisconsin. Pp. 220. Price $6.00.

A Symposium on Pest Control and Wildlife Relationships. Publication 897 of the National Academy of Sciences—National Research Council, Washington, D. C. Pp. 25.

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